In Vitro Assessment of Humoral Immunity Following Exposure to Heavy Metals

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The immune system of animals and man is extremely complex. This report will discuss the effect metals have on one segment of the immune system; that is, humoral immunity. Humoral immunity is essentially the production of antibody in response to an antigen. The B-lymphocyte is the primary cell responsible for producing antibody. However, this cell is regulated by T-lymphocytes and macrophages.

Many methods are available to assess humoral immune responses. A multitude of immunoassays have been developed for enumeration of serum antibody. Some of these are immunodiffusion, complement fixation, serum neutralization, hemagglutination, radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). Detection of antibody-forming cells distinguishes between effect on antibody production compared to degradation of preformed antibody. Other available methods are measurement of surface receptor (Fc and complement) activity on B-cells. Mitogens (T-independent) have also been regarded as a measurement for humoral immunity.

In our laboratory, we have developed an ELISA technique that is appropriate for general assessment of humoral immune responses resulting from chemical exposure. This technique is highly sensitive and can be mechanized. The assay can easily be incorporated into the drug and chemical efficacy testing programs of industry.

The immune system of animals and man is complex and cooperation between the three major components, humoral, cell-mediated and macrophage systems, is often necessary for optimal expression. The major identifying property of humoral immunity is the production and secretion of antibody by lymphocytes. The B-lymphocyte is the cell directly responsible for humoral immunity by differentiation into antibody producing (plasma) cells after antigen stimulation.

Introduction of antigen into a host results in either direct stimulation of B-cells or the antigen may initially be phagocytized by macrophages which transfers the antigenic determinants to B-lymphocytes. The sensitized small lymphocytes progress through a series of transformations into large lymphocytes and finally lymphoblasts, which differentiate into antibody-secreting plasma cells or small memory lymphocytes. The reaction commences when antigen molecules encounter small lymphocytes with surface antibody to that particular antigen. The antigen is bound to the antibody which provokes the small lymphocytes to divide and differentiate. During differentiation, the surface antigen–antibody complexes are lost and replaced by new antibody receptors. The memory cells possess surface immunoglobulin receptors similar to the original small lymphocytes while plasma cells synthesize and secrete circulating antibody.

The memory cell, on the other hand, is a resting antigen-sensitized B-cell which responds to a second exposure of that antigen by rapid production

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of large amounts of antibody. These antibodies usually have a greater affinity and specificity for antigen than those produced after initial exposure.

Antibody responses to many antigens require cooperation between at least two types of lymphocytes for optimal expression. Differentiation of bone marrow-derived B-cells into antibody-secreting plasma cells, is usually regulated by thymus-derived T-cells. The function of the T-cell and its soluble factors is not to produce antibody, but rather to aid in activation and proliferation of B-cells (1). A third cell type, the macrophage, also has an important role as an accessory cell by cooperating with T-cells and aiding the response of B-cells to antigens.

There are at least four types of T-cells recognized to date. These include cytotoxic, helper, amplifier and suppressor T-cells. All but the cytotoxic lymphocyte function in regulating the humoral immune response. The helper T-cell is required for activation of B-cells to many antigens, while the amplifier T-cell regulates proliferation of activated B-lymphocytes. This cooperation may occur either by cell to cell contact or by release of soluble factors by T-cells (2). The other T-cell, suppressor T, is responsible for suppressing antibody synthesis by inhibiting B-lymphocytes. In general, B-cell activity is regulated by a balance of helper and suppressor T-cells. Exposure to a weak or small antigen dose may induce helper T-cells, while a strong antigen or larger dose may induce suppressor T-cells, thereby controlling amount of antibody synthesized.

Macrophages are also modulators of humoral immunity. Macrophages, like B-lymphocytes, have receptors for complement and the Fc portion of immunoglobulin. One function of macrophages is to localize antigen and bring it closer to an antibody-forming lymphocyte. Macrophages may also process antigen in lysosomes making it highly antigenic (3-5). Like the T–B cell interaction, this cooperation may occur by physical contact between the macrophage and B-cell or by soluble factors released from the macrophage. Macrophages also activate T-lymphocytes to produce nonspecific helper activity for antibody production (6, 7).

In summary, the pathway of the humoral immune system from antigen stimulation to antibody synthesis is extremely complex. The B-lymphocyte is the progenitor of antibody-forming plasma cells, but optimum responses are not usually achieved without participation by T-lymphocytes and macrophages. Furthermore, it is a balance between helper T and suppressor T as well as amplifier T and macrophages that regulate B-cell activity and consequently the humoral immune response.

Immunoassays used to examine humoral immunity include those which measure antibody titers (immunodiffusion, complement fixation, serum neutralization, hemagglutination, passive hemagglutination, radioimmunoassay, enzyme-linked immunosorbent assay, etc.), antibody synthesis (Jerne plaque) and B-lymphocyte receptors (EA = Fc and EAC = C3). Humoral immunity may be determined after initial exposure to the antigen (primary response) or after rechallenge with antigen (secondary or memory response). Staining of surface immunoglobulin will identify B-lymphocytes and enumerate percent B-cells. Many antigens, both T-dependent and T-independent, have been utilized in these investigations. Some of these antigens are tetanus toxoid, sheep red blood cells, bovine serum albumin, bovine gammaglobulin, *Salmonella typhi*, pseudorabies virus, influenza virus, keyhold limpet hemocyanin and lipopolysaccharide.

Metals and metal compounds responsible for reduced circulating antibody titers are lead (8, 9), cadmium (9, 10), methylmercury (9, 11, 12), arsenic (12), nickel (13), chromium (13), and platinum (14). Antibody synthesis has been inhibited by lead (12, 15-17), cadmium (17, 18-20), methylmercury (12, 21, 22), zinc (23), arsenic (12), silica (24) and magnesium (25). Selenium, on the other hand, has stimulated both antibody synthesis and circulating antibody titers (26-28). Lead and cadmium (29) inhibit the activity of B-lymphocyte receptors, while methylmercury (30) actually augments the complement receptor activity on B-cells. Finally, lead, cadmium and methylmercury have been demonstrated to affect immunological memory (31).

It has been well documented that many of these metals compromise the immune system of experimental animals. Damage may occur to a particular cell (B, T or macrophage) or may involve more than one cell which regulates the proliferation and differentiation of other cells responsible for normal function of the immune system. We have accepted the ELISA as the test procedure of choice in our laboratory for humoral immunity. This assay is simple to perform, reliable, highly sensitive, economically feasible, has minimum variation, requires only a few test animals and can be mechanized. Further, in all of our investigations of environmental chemicals in which antibody titers were decreased, antibody synthesis was also inhibited; an indication that the more complex antibody synthesis techniques are unnecessary to perform except for special instances. Therefore, we are confident that the ELISA technique is appropriate for general assessment of humoral immune responses in animals that have been exposed to chemicals.

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